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INVESTIGATION OF THERMOPHILIC MICROFLORA FOR THE SELECTION OF STARTER CULTURE COMPOSITION FOR A FUNCTIONAL PRODUCT

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Fermented dairy products have valuable nutrients and contain a large number of living cells of lactic acid bacteria strains of lactic acid bacteria. The selection of microorganisms according to their technological and microbiological properties and their ability to develop in a dairy base, as well as organoleptic and rheological parameters of the finished fermented milk product, is an important stage in the development of new types of products. Therefore, special attention was paid to the selection of starter cultures for the production of a functional product. The authors conducted research on the selection of starter culture microflora for the production of functional products. The microbiological and organoleptic properties of selected microorganisms have been studied. When selecting microorganisms, the intensity and direction of the microbiological and biochemical processes occurring during fermentation were taken into account, which is determined by the development of the starter microflora. Thermophilic starter culture by species *Lactobacillus acidophilus, Streptococcus thermophiles, Lactobacillus bulgaricus* was used in the work.

Key words: fermented milk product, microflora, technology, functional fermented milk products, fermentation.

ФУНКЦИОНАЛДЫ ӨНІМГЕ АРНАЛҒАН АШЫТҚЫ ҚҰРАМЫН ТАҢДАУ ҮШІН ТЕРМОФИЛЬДІ МИКРОФЛОРАНЫ ЗЕРТТЕУ

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Сүт қышқылды өнімдердің құнды қоректік заттарға бай болуы құрамындағы сүт қышқылы бактериялардың штамдарының тірі жасушаларына байланысты. Өнімнің жаңа түрлерін алуда маңызды кезең болып, микроорганизмдерді технологиялық, микробиологиялық қасиеттері бойынша, сондай-ақ, сүт қышқылы өнімінің органолептикалық және реологиялық көрсеткіштерін таңдау негіз болып табылады. Осы тұрғыда ашытқыларды таңдау функционалды өнімді өндіру үшін ерекше назарды аударады. Статьяда авторлар функционалды өнімдерді өндіру үшін ашытқы микрофлорасын таңдау бойынша зерттеулер жүргізді. Таңдалған микроорганизмдердің микробиологиялық және органолептикалық қасиеттері зерттелді. Микроорганизмдерді таңдауда ашыту кезінде жүретін микробиологиялық, биохимиялық процестердің қарқындылығы мен бағыты ескерілді, бұл ашытқы микрофлорасының дамуымен анықталған. Жұмыста термофильді ашытқы культуралары - *Lactobacillus acidophilus, Streptococcus thermophiles, Lactobacillus bulgaricus* түрлері қолданылды.

Түйін сөздер: сүт қышқылды өнім, микрофлора, технология, функционалды сүт қышқылды өнім, ферментация.

ИССЛЕДОВАНИЕ ТЕРМОФИЛЬНОЙ МИКРОФЛОРЫ ДЛЯ ПОДБОРА СОСТАВА ЗАКВАСКИ ДЛЯ ФУНКЦИОНАЛЬНОГО ПРОДУКТА

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Кисломолочные продукты обладают ценными питательными веществами и содержат большое количество живых клеток молочнокислых бактерий штаммов молочнокислых бактерий. Подбор микроорганизмов по технологическим и микробиологическим свойствам и по способности их развиваться в молочной основе, а также по органолептическим и реологическим показателям готового кисломолочного продукта, является важным этапом в разработке новых видов продуктов. Поэтому подбору заквасок для производства функционального продукта было уделено особое внимание. Авторами проведены исследования по подбору заквасочной микрофлоры для производства функциональных продуктов. Изучены микробиологические и органолептические свойства подобранных микроорганизмов. При подборе микроорганизмов учитывались интенсивность и направленность микробиологических, биохимических процессов, протекающих во время ферментации, что определяется развитием заквасочной микрофлоры. В работе использовалась термофильная заквасочная культура по видам *Lactobacillus acidophilus, Streptococcus thermophiles, Lactobacillus bulgaricum.*

Ключевые слова: кисломолочный продукт, микрофлора, технология, функциональные кисломолочные продукты, ферментация.

Introduction. Fermented milk products occupy a special place in the human diet and are in great consumer demand due to their taste and dietary properties. They contain all the necessary substances that ensure normal human functioning, are easily digested, contribute to the easy process of digesting food, regulate and, if necessary, restore intestinal microbiocenosis.

The technology of functional dairy-based food products involves the fermentation of raw materials by specially selected probiotic microorganisms. In this work, microorganisms obtained from manufacturers of starter cultures of «Propionix» LLC (Russia) and MicroMilk company (Italy) are used for this purpose.

The composition and properties of starter cultures, which provide specific organoleptic and biological properties, have a certain effect on the indicators of functional fermented milk products [1].

Industrial probiotic products have a number of objective disadvantages from a therapeutic point of view. This circumstance, combined with the high stability of the established microbiocenoses, is a factor determining the need for long-term intake of adequate doses of probiotic products. To enhance the therapeutic effect of the product, it is necessary to use several strains of microorganisms. According to many researchers, multi-strain starter cultures are resistant to adverse environmental factors and have higher activity compared to starter cultures prepared on monocultures [2, 3].

The main purpose of the work was the selection of thermophilic microflora for the preparation of a starter culture for a protein product according to the biological and organoleptic properties of microorganisms and the study of the quality of the formed clot after the fermentation process.

Materials and methods. To implement the tasks set in the work, experimental studies were conducted in the laboratories of the Department of Technology and Standardization of the Kazakh University of Technology and Business.

The objects of the study were:

- skimmed cow's milk according to GOST 13264 obtained by separating the harvested milk, with an acidity of not more than 20 °T, with a density of 1029 kg / m^3.

Strains have been studied as biological objects:

- Lactobacillus bulgaricum Bulgarian wand YO100 (10U) manufactured by MicroMilk, Italy.

- Lactobacillus acidophilus. MicroMilk company, Italy.

- Streptococcus thermophilus, Danisco France SAS, France.

When performing the work, generally accepted, standard methods of research of physico-chemical and microbiological parameters of raw materials and finished products, sensory indicators were used. The following research methods were used in the work:

- sampling and preparation for testing according to GOST 26809-86;

- the density of skimmed milk according to GOST 3625-84;

- acidity by titrimetric method according to GOST 3624-92;

- microscopy of microorganisms. Photos of micro-paintings of bacteria, microscopy of the finished product were taken using an electronic digital microscope according to GOST 9225-84;

- methods for the determination of lactic acid microorganisms "Food products" according to GOST 10444.11-89.

The experiments were carried out in three- and five-fold repetition.

Results and discussion. The selection is based on general microbiological criteria: safety, manufacturability, positive effect on health and properties of the strain. When selecting probiotic microorganisms from collection strains, we select the following type of energetic acid-forming agents: *Str.thermophilus, L.acidophilus, L.bulgaricum.* These cultures are classified as probiotics that have a regulating and stimulating effect on the body and are not antagonistic to each other, producing metabolic products that play an important role in the functioning of the human body. The introduction of energetic acid-forming agents into the polyzaccharacter contributes to a significant accumulation of lactic acid and the production of a dense clot with intensive separation of serum [4].

Resistance to lysozyme, gastric juice, as well as adhesive properties, and the production of antibiotic substances play an important role. Lactobacilli are actively involved in the processes of proteolysis. In this case, the protein is converted into easily digestible components (proteinases and lactobacilli), which in turn form polypeptides [5].

Lactic acid bacteria need nutrients in the form of amino acids and peptides for their development. B vitamins are the most essential for the vital activity of most species, thiamine is mainly needed for heterofermentative lactobacilli, biotin and vitamin B_{12} – only for some strains. The requirements for folic acid, riboflavin, pyridoxal phosphate, and para-aminobenzoic acid differ from species to species [6].

An important conclusion was made by VNMI researchers when comparing the clinical results obtained using probiotic products and bacterial concentrates. The therapeutic efficacy of probiotic products is higher compared to bacterial concentrates, this circumstance is due to the large volume of the medium containing the metabolic products of probiotic microorganisms [7, 8].

From a technological point of view, rheological properties and organoleptic characteristics of the selected microflora are important for production.

In addition to the biochemical properties, the identified strains of *Str. thermophilus, L.acidophilus, L.bulgaricum* were selected on the basis of production qualities, the most important being the taste, smell and consistency that the strains form in their fermented milk. The next condition for the selection of strains is the existence of a symbiotic relationship between them. Researchers explain the symbiotic relationship between *L.bulgaricum* and *Str. thermophilus* by the peculiarities of their metabolism, nutritional needs and development conditions. *L.bulgaricum* has a pronounced proteolytic activity characterized by a set and amount of free amino acids accumulated during its development in milk. *Str. thermophilus* has weak proteolytic activity in milk and forms only proline from amino acids, of which there are only traces in fresh milk and consumes all other amino acids. *Str. thermophilus* uses all amino acids for its development, especially essential ones and valine produced by *L. bulgaricum.*

Str. thermophilus is ahead of the Bulgarian bacillus in its development, reducing the redox potential and pH, thereby creating more favorable conditions for the development of *L. bulgaricum.* According to Bulgarian scientists, *Str. thermophilus* begins to develop for the first time in 30 minutes, the number of cells increases and reaches a maximum at the time of complete coagulation of milk after 2.5 hours. Its dying phase begins 9-10 hours after fermentation. The Bulgarian bacillus begins to multiply an hour after the introduction of the starter culture and reaches its maximum just an hour after the *Str. Thermophilus* has reached its maximum [9, 10].

The qualitative characteristics of the microflora are presented in Table 1.

Table 1 -Qualitative characteristics of microflora

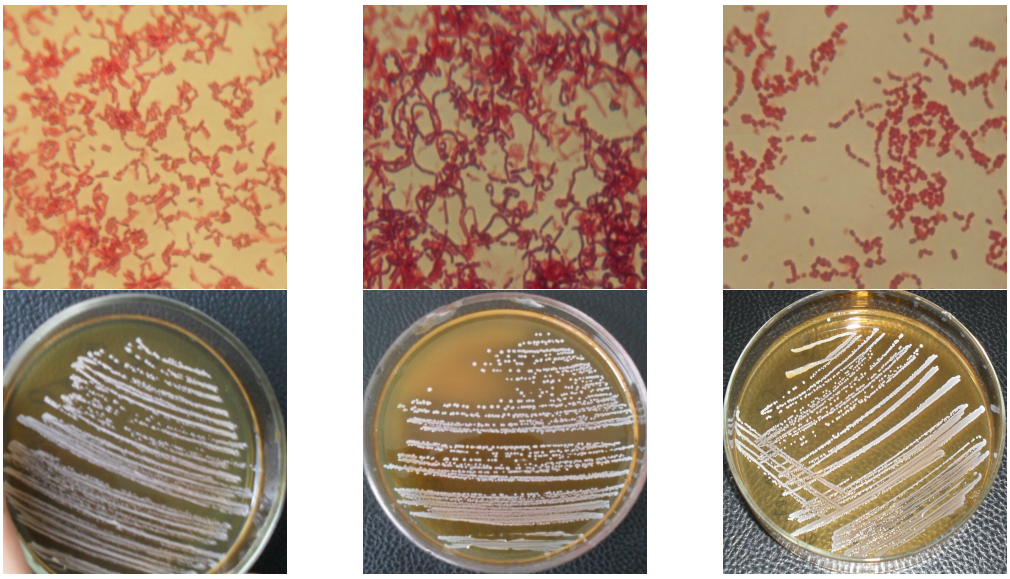
Microorganisms	Fermentation time, h	Fermentation temperature, °C	Titrated acidity, °T
Lactobacillus bulgaricum	3-5	37	118-250
Streptococcus thermophilus	5-7	37	110-130
Lactobacillus acidophilus	4-6	37	170-250

The table shows that the selected microorganisms have the same fermentation temperature characteristic of thermophilic microorganisms.

In production, the quality of fermented milk products largely depends on the starter culture used, which provides them with specific organoleptic, biological, and functional properties.

The incubation of microorganisms was carried out at a temperature of +37 ° C for 48 hours according to the developed instructions for the preparation of starter cultures.

The purity of the strains was checked by microscopy of the preparations (Fig. 1). To prepare the drug, a small drop of the test material was applied to a clean slide with a loop and distributed over an area of about 1 cm^2. The preparation was dried at room temperature, fixed on a burner flame and painted.



Lactobacillus bulgaricus Lactobacillus acidophilus Streptococcus thermophilus

Figure 1 - Microscopy of the studied strains

The microflora was seeded with a debilitating stroke on dense media. Microscopy of the strains showed the presence of cocci collected in chains of different lengths of gram-positive cells and rod-shaped (rods are straight, large, single and in chains) [6].

Selected cultures are able to form extracellular polymers that are carbohydrate-protein complexes, the carbohydrate part of which includes glucose, galactose, rhamnose, and a number of amino acids in the protein part. The use of such starter cultures, selected according to the ability of its microflora to produce extracellular polymers, provides a significant improvement in product quality [11].

The products of carbohydrate fermentation are acetic, lactic, propionic and butyric acids, which are useful for humans, since the pH in the large intestine decreases, which leads to the destruction of pathogenic microorganisms and the cessation of the formation of protein decay products, better absorption of micro- and macronutrients, primarily calcium and iron [12].

After incubation, the titrated acidity of the clots and organoleptic parameters were studied, the results are shown in Table 2.

Table 2– Organoleptic parameters of the clot

Microorganisms	Titrated acidity, °T	Organoleptic indicators
Str. thermophilus	80-90	the taste is sharp (spicy), fermented milk, without foreign tastes and odors, the consistency of the clot is homogeneous, coarseness is allowed, a small separation of serum is noted
L. bulgaricum	100-130	the taste and smell are clean, fermented milk, without foreign tastes and odors, the consistency of the clot is homogeneous
L. acidophilus	100-130	the taste is prickly, sour and the smell is clean, fermented milk, without foreign tastes and odors, the consistency of the clot is homogeneous, viscous, mucosity is allowed

Figure 2 - Organoleptic parameters of the clot

During fermentation, it must be borne in mind that milk contains all the nutrients necessary for the development of heterotrophic microorganisms: lactose – about 4.5%, proteins – 5%, mineral compounds – 1%, vitamins. Milk without additives contains approximately 0.01% of free amino acids, which is less than 20% of the amino acids found in environments that ensure optimal bacterial growth. In order to achieve normal growth in an environment with milk casein as the main source of nitrogen, organisms must have a certain ability to proteolysis.

Conclusions. Thus, it can be concluded that the selected associate of microorganisms has the following advantages: when cultivated, the selected microorganisms exhibit high acid resistance; when cultivated separately, *L.bulgaricum* and *Str. thermophilus* quickly lose their characteristic morphological properties and degenerate, while when cultivated together they retain these qualities for a long period; form a right-rotating (L+) the shape and mixture of isomers resistant to adverse environmental factors compared to monocultures, and have high biochemical activity.

It is assumed that the joint cultivation of microorganisms will give a more accelerated fermentation, since during prolonged fermentation, simultaneously with the development of the microflora of starter cultures, a conditionally pathogenic microflora or bacteriophage multiplies; the use of the strain *Str.thermophilus*, which has increased activity to produce β-galactosidase, and the strain *L. bulgaricum*, which produces L(+) lactic acid in a dominant amount, which is physiological for the human body.

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